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## Adaptor Function of PapF Depends on Donor Strand Exchange in P-Pilus Biogenesis of *Escherichia coli*<sup>▽</sup>

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**P-pilus biogenesis occurs via the highly conserved chaperone-usher pathway and involves the strict coordination of multiple subunit proteins. All nonadhesin structural P-pilus subunits possess the same topology, consisting of two domains: an incomplete immunoglobulin-like fold (pilin body) and an N-terminal extension. Pilus subunits form interactions with one another through donor strand exchange, occurring at the usher, in which the N-terminal extension of an incoming subunit completes the pilin body of the preceding subunit, allowing the incorporation of the subunit into the pilus fiber. In this study, pilus subunits in which the N-terminal extension was either deleted or swapped with that of another subunit were used to examine the role of each domain of PapF in functions involving donor strand exchange and hierarchical assembly. We found that the N-terminal extension of PapF is required to adapt the PapG adhesin to the tip of the fiber. The pilin body of PapF is required to efficiently initiate assembly of the remainder of the pilus, with the assistance of the N-terminal extension. Thus, distinct functions were assigned to each region of the PapF subunit. In conclusion, all pilin subunits possess the same overall architectural topology; however, each N-terminal extension and pilin body has specific functions in pilus biogenesis.**

P pili are adhesive organelles that are critical virulence factors in the establishment of pyelonephritis by uropathogenic *Escherichia coli* (23, 36–38). These adhesive organelles are macromolecular, fibrous structures on the surface of the bacteria that mediate the recognition of and attachment to tissues of the kidney by the pathogen (5, 17, 23, 29, 31, 34, 36–38, 42). This attachment is a key step in the pathogenic cascade of uropathogenic *E. coli*, leading to the establishment of infection (36, 38). Assembly of pilus fibers requires the coordination of at least 11 structural and regulatory genes contained in the *pap* gene cluster (33, 35) and proceeds via the chaperone-usher pathway (43). This pathway is utilized by a wide variety of gram-negative bacteria to produce more than 100 different adhesive organelles, of which the P pilus is considered a prototypical member (15, 19; unpublished data).

P pili have a multicomponent architecture, consisting of a thin tip fibrillum and a thicker cylindrical rod that are each composed of multiple subunits (PapG, PapF, PapE, PapK, and PapA) (Fig. 1A) (2, 5–7, 11, 13, 21, 23, 25). Pili are assembled in a top-down fashion as they emerge from the bacterial cell surface (43), and the adhesin PapG is localized to the distal tip of the fiber (Fig. 1A) (25, 31). The order of the remaining pilus subunits has been delineated through a combination of biochemical studies and electron microscopy (2, 6, 7, 13, 21, 23, 25). The tip fibrillum is thought to be composed of PapG, PapF, and PapE (Fig. 1A) (21, 25, 32). It is a flexible structure

consisting mainly of repeating PapE subunits interacting linearly with each other to form an approximately 2-nm-diameter polymer (21, 25). PapF is thought to connect PapG to the PapE polymer (21, 32, 34), and the entire tip fibrillum is connected via the PapK adaptor protein (21) to the rigid pilus rod consisting of PapA subunits (2) (Fig. 1A). The thick rod contains PapA subunits arranged to form a right-handed helical cylinder with 3.28 subunits per turn (2, 7, 13). Finally, the PapH subunit is thought to terminate assembly and anchor the entire composite structure to the outer membrane (1) (Fig. 1A).

Each nonadhesin subunit in the pilus fiber contains two domains, each of which has the same overall topology in all subunits: an N-terminal extension and an incomplete immunoglobulin (Ig)-like fold (pilin body) (39, 40) (Fig. 1A). Instead of an N-terminal extension, the adhesin subunit has a receptor-binding domain connected to the pilin body domain (9, 11). The N-terminal extensions each contain a conserved motif of alternating hydrophobic residues, including an invariant glycine (39, 40) (Fig. 1B). A conserved cysteine demarcates the boundary between this domain and the first A1 beta strand of the pilin body (39, 40) (Fig. 1B). The pilin body of each subunit lacks the final seventh beta strand of the canonical Ig fold (3, 9, 39, 40). The missing strand exposes the hydrophobic core and renders each of the subunits highly unstable, targeting it for rapid proteolytic degradation in the periplasmic space if it is not coproduced with the PapD chaperone (3, 9, 22, 28, 39, 40). The chaperone consists of two complete Ig-like domains (3, 9, 14, 18–20, 27, 39, 40). In order to stabilize the subunits, the chaperone donates the G1 beta strand to complete the fold of the subunit, inserting parallel to the F beta strand of the pilin body in donor strand complementation (3, 9, 39, 40).

In donor strand exchange, the N-terminal extension of an incoming pilus subunit displaces the G1 beta strand of PapD

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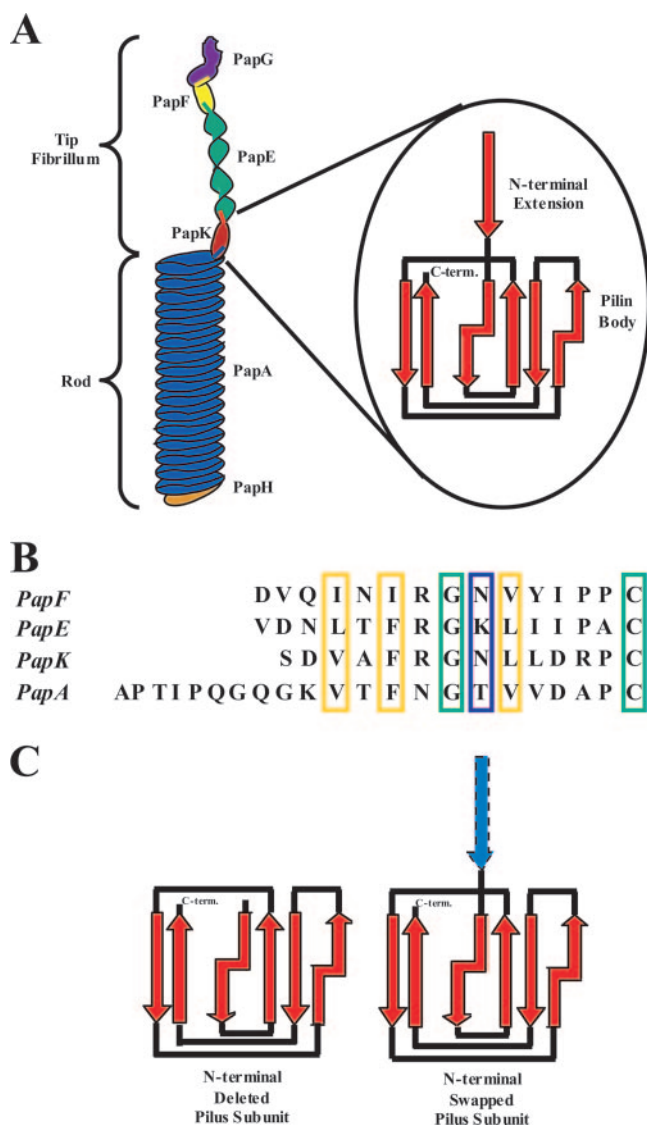


FIG. 1. Structural P-pilus subunits have the same overall topology and are assembled in a specific order in the fiber. (A) Schematic diagram of the P pilus, showing the arrangement of subunits. P pili consist of a thin tip fibrillum and a thicker rod. The adhesin PapG (purple) localizes to the distal tip of the fiber and is connected to the remainder of the pilus through PapF (yellow). PapE (green) forms a linear polymer, making up the bulk of the tip fibrillum, which is connected to the PapA (blue) rod via PapK (red). PapH (orange) is thought to anchor the fiber to the bacterial cell membrane. Also illustrated is a diagram of a pilus structural subunit. Each nonadhesin structural subunit of the P pilus contains an N-terminal extension preceding an incomplete Ig-like fold (pilin body). (B) Alignment of the N-terminal extensions. An alignment of the amino termini shows a conserved motif of alternating hydrophobic residues (yellow boxes), including an invariant glycine (green box). An invariant cysteine (green box) marks the boundary between this domain and the beginning of the Ig-like fold. (C) Schematic diagram of Ntd and Nts mutants used in this work. In Ntd subunits, the N-terminal extension is removed, leaving behind an intact incomplete Ig-like domain. In contrast, the N-terminal extension of one subunit is replaced by the same region of another subunit in Nts subunits, regenerating a subunit containing both domains.

from a neighboring subunit (3, 4, 9, 39, 40) and inserts antiparallel to the subunit C-terminal F beta strand, creating a stable canonical Ig-like fold (9, 39, 40). These tight interactions are thought to give the fiber its extraordinary stability (9, 39, 40). The process of donor strand exchange depends on the PapC usher (40). The usher is a twin-pore complex with 2- to 3-nm channels (30, 44). Each of the chaperone-subunit complexes is targeted in turn to the usher, beginning with the PapD-PapG chaperone-adhesin complex, which has the highest affinity for PapC (10, 41, 44). Following binding to the usher, the chaperone in each complex dissociates from the pilus subunit (18), exposing the underlying interactive surfaces of the subunit (26, 40) and allowing donor strand exchange to proceed (4, 9, 18, 39, 40).

Each subunit has the same overall topology and undergoes the same assembly processes via the chaperone-usher pathway as the other subunits (9, 39, 40), leading to speculation that each subunit is functionally equivalent to the others. However, recent investigations into the activation of the CpxRA two-component pathway by P-pilus assembly revealed that various subunits each have distinct activities (28). Furthermore, early characterization of PapF suggested that it has unique adaptor and initiator roles in pilus biogenesis (21, 32). *E. coli* K-12 strains carrying a *papF* mutant operon, constructed from the *E. coli* J96 *pap* operon, exhibited severe pilus assembly defects compared to strains carrying a wild-type *pap* operon (21). This phenotype was found to be unique to strains with mutations in *papF* (21). Strains deficient in PapF also showed an inability to agglutinate human red blood cells, an activity mediated by the PapG adhesin (21). The basis for these properties of PapF remains elusive.

In this study, the correlation between the arrangement and function of subunits in the pilus fiber was investigated. N-terminal deleted (Ntd) and N-terminal swapped (Nts) subunits were used to examine and alter the order of subunit incorporation in the fibers. We found that the PapF N-terminal extension was necessary for PapF to function as the PapG adaptor protein, while the specific PapF pilin body was not required for interactions with PapG. In contrast, the specific PapF pilin body was necessary for PapF to interact with PapE, as well as to actively participate in the initiation of the assembly of the remainder of the pilus structure. These studies demonstrate that each domain of each subunit is distinct from the others in function. Thus, characterization of the role of PapF in the prototypical P-pilus system may provide insight into assembly events that may also occur in the biogenesis of a multitude of other adhesive fibers.

#### MATERIALS AND METHODS

**Bacterial strains and genetic constructs.** All bacterial strains used in this study are listed in Table 1. *E. coli* laboratory strain MC4100 (8), which does not carry the *pap* operon, was used as the host strain in all studies except in cloning steps. Cloning and subcloning were done with *E. coli* laboratory strains MC1061 (45) and TOP10 (Invitrogen).

Plasmid pYML76 carrying the *papE* mutant operon under control of the natural promoter was made by moving the EcoRI-BamHI fragment encoding the *papE* mutant operon from pPAP15 (33) into pACYC184 (NEB). A blunt end was generated at the BamHI site of the *papE* mutant fragment prior to ligation into a pACYC184 vector that was cut with EcoRI and ScaI. The following plasmids have been previously described elsewhere. Plasmid pPAP15 contains a *papE* mutant operon in which *papE* was disrupted by insertion of an XhoI linker (33). pFJ3 contains the *pap* operon under control of the native promoter in



TABLE 1. Strains and plasmids used in this work

Strain or plasmid	Description	Reference or source
<b>Strains</b>		
MC4100	F <sup>-</sup> <i>araD139</i> $\Delta$ ( <i>argF-lac</i> ) <i>U169 rpsL150</i> (Str <sup>r</sup> ) <i>relA1 flbB5301 deoC1 ptsF25 rbsR</i>	8
MC1061	F <sup>-</sup> <i>araD139</i> $\Delta$ ( <i>ara-leu</i> )7696 <i>galE15 galK16</i> $\Delta$ ( <i>lac</i> )X74 <i>rpsL</i> (Str <sup>r</sup> ) <i>hsdR2</i> ( <i>r<sub>K</sub></i> <sup>-</sup> <i>m<sub>K</sub></i> <sup>+</sup> ) <i>mcrA mcrB1</i>	45
TOP10	F <sup>-</sup> <i>mcrA</i> $\Delta$ ( <i>mrr-hsdRMS-mcrBC</i> ) $\phi$ 80 <i>lacZ</i> $\Delta$ M15 $\Delta$ <i>lacX74 recA1</i> <i>araD139</i> $\Delta$ ( <i>ara-leu</i> )7697 <i>galU galK rpsL</i> (Str <sup>r</sup> ) <i>endA1 nupG</i>	Invitrogen
<b>Plasmids</b>		
pMMB66	Expression vector	12
pACYC184	Expression vector	NEB
pPAP15	<i>papE</i> mutant operon	33
pFJ3	Wild-type <i>pap</i> operon (natural promoter)	21
pFJ9	<i>papF</i> mutant operon (natural promoter)	21
pHJ13	Wild-type PapE expression plasmid	22
pYML36	NtdPapE expression plasmid	28
pYML43	Wild-type PapF expression plasmid	28
pYML55	Nts <sub>E</sub> PapF expression plasmid	28
pYML56	Nts <sub>F</sub> PapE expression plasmid	28
pYML64	NtdPapF expression plasmid	28
pYML75	Nts <sub>E</sub> PapK expression plasmid	28
pYML76	<i>papE</i> mutant operon (natural promoter)	This study

pACYC184 (21). pFJ9 contains a *papF* mutant operon under control of its natural promoter, in which *papF* was disrupted by insertion (21). pHJ13 expresses wild-type *papE* under control of the inducible *P<sub>tac</sub>* promoter in the vector pMMB66 (22). pYML43 encodes wild-type *papF* under control of the inducible *P<sub>tac</sub>* promoter in pMMB66 (28). pYML36 encodes NtdPapE (28), and pYML64 contains NtdPapF in pMMB66 (28). pYML55 encodes the PapF pilin body with an N-terminal extension of PapE (Nts<sub>E</sub>PapF) (28), and pYML56 encodes Nts<sub>F</sub>PapE in the pMMB66 parent vector background (28). pYML75 encodes Nts<sub>E</sub>PapK in pMMB66 (28).

**HA assays.** Hemagglutination (HA) assays were performed essentially as previously described (21), except that pilus production was induced by one growth passage instead of three. This was done to inhibit accumulation of mutants or revertants due to the overproduction of toxic wild-type (e.g., PapE) or mutant subunits. Briefly, a starter bacterial culture was grown to saturation overnight in LB broth in the presence of antibiotics appropriate for the strain. A 500- $\mu$ l portion of the saturated overnight culture was then spread on large petri dishes (150 by 15 mm) containing solid tryptic soy agar (for induction of pili from the native promoter) supplemented with both 0.001 mM (final concentration) isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) (for induction of subunits from the *P<sub>tac</sub>* promoter) and appropriate antibiotics. The strains were incubated overnight at 37°C. Cells were then scraped from the petri dishes and resuspended in 1 $\times$  phosphate-buffered saline (PBS) (pH 7.4) (Sigma) to an optical density at 540 nm of  $\sim$ 1.0. The bacteria were then concentrated 10-fold by centrifugation of 1 ml of each cell suspension and resuspension of the pellet in 100  $\mu$ l of fresh PBS. The concentrated suspension was then serially diluted twofold in a 96-well V-bottom plate (Costar). Human red blood cells were washed in PBS and then suspended in PBS to an absorbance at 640 nm of  $\sim$ 1.6. Equal volumes of the blood were then added to the wells of the 96-well plate, and the plate was covered and incubated for 1 h at 4°C. The HA titer was defined as the first dilution at which agglutination was no longer observed. Each experiment was done in triplicate, and the titers were averaged.

**Pilus production.** Piliated bacterial cells were grown as described above for the HA assays. The cells were suspended in PBS to an optical density at 540 nm of  $\sim$ 1.0. The cells were then washed twice in PBS, and the final pellet was resuspended in an equal volume of fresh PBS. Negative-stain electron microscopy was then done as previously described, using 1% uranyl acetate for staining (21, 24).

Populations of 200 bacterial cells per strain were observed and catalogued based on the amounts of pili produced, as follows: no pili (bald), low numbers of pili (<20 pili per cell), moderate numbers of pili (intermediate levels), and abundant pili (high levels). The data were expressed as the percentages of the total cells in each category.

**Coomassie blue and immunoblot analyses of pilus and periplasmic preparations.** Bacterial cells were grown as described above for the HA assays. Cells were scraped from the plates, weighed, and resuspended in 1 $\times$  PBS (5 ml per g of cells). Separate aliquots of each cell suspension were then subjected to either isolation of the pili or extraction of the periplasmic contents. For pilus preparations, resuspended cells were heated at 65°C for 1 h to release P pili (21). Depiliated cells were then pelleted by centrifugation, and the resultant crude pilus preparations were incubated in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) sample buffer supplemented with 4 M urea at 95°C for 5 min prior to analysis by SDS-PAGE followed by Coomassie blue staining or by immunoblotting using anti-PapGI pilin antibodies in conjunction with anti-rabbit immunoglobulin G-alkaline phosphatase conjugate secondary antibodies. Immunoblots were developed using the 5-bromo-4-chloro-3-indolyl-phosphate (BCIP)-nitroblue tetrazolium substrate system (Sigma). Periplasm was prepared by the lysozyme-EDTA method as previously described (10) and subjected to immunoblotting as described above.

# RESULTS

**The PapF N-terminal extension is required for proper PapG presentation.** Jacob-Dubuisson et al. demonstrated that PapF is required for *E. coli* expressing P pili to agglutinate human red blood cells (21), presumably as a result of its role in adapting PapG to the remainder of the pilus fiber. To understand the molecular basis for the function of PapF as an adaptor protein, the ability of Ntd and Nts (Fig. 1C) PapF and PapE subunits to complement a *pap* gene cluster lacking *papF* in HA assays was tested. Ntd subunits lack the majority of the amino-terminal extensions but retain the signal sequences and pilin bodies (Fig. 1C) (28, 40). In Nts subunits, the complete N-terminal extension of one subunit is exchanged with that of another subunit, without modification of the pilin body of the subunit (Fig. 1C) (28). The Ntd and Nts constructs therefore enable examination of each specific domain of the pilus subunits (the N-terminal extension or the pilin body domains) in functional assays of pilus biogenesis, such as HA assays. *E. coli* cells expressing pili from the wild-type *pap* operon are able to agglutinate human red blood cells (18, 21). Agglutination results from the interaction of the PapG adhesin with moieties on the red blood cells, and thus, the HA assay provides a measure of the correct incorporation and proper presentation of PapG at the end of the tip fibrillum (18, 21). Therefore, Ntd and Nts subunits were used as tools in HA assays to determine whether the presentation of PapG could be altered by the presence or absence of the specific PapF N-terminal extension or pilin body.

In these assays, *E. coli* laboratory strain MC4100 was used because the *pap* operon is not present in the genome of this bacterium. A PapF<sup>-</sup> strain was constructed by transforming pFJ9, a plasmid with a *papF* mutant *pap* operon under control of the natural promoter (21), into MC4100. Wild-type PapF, wild-type PapE, NtdPapF, NtdPapE, Nts<sub>F</sub>PapE, Nts<sub>E</sub>PapF, or an empty vector (pMMB66) was then supplied in *trans*, and the strains were tested to determine their abilities to agglutinate red blood cells. MC4100 carrying the entire wild-type *pap* operon and MC4100 carrying an empty vector (pACYC184) were also examined to establish producing and nonproducing levels of HA titers for comparison in each experiment. *E. coli* MC4100 cells carrying the wild-type *pap* operon produced a

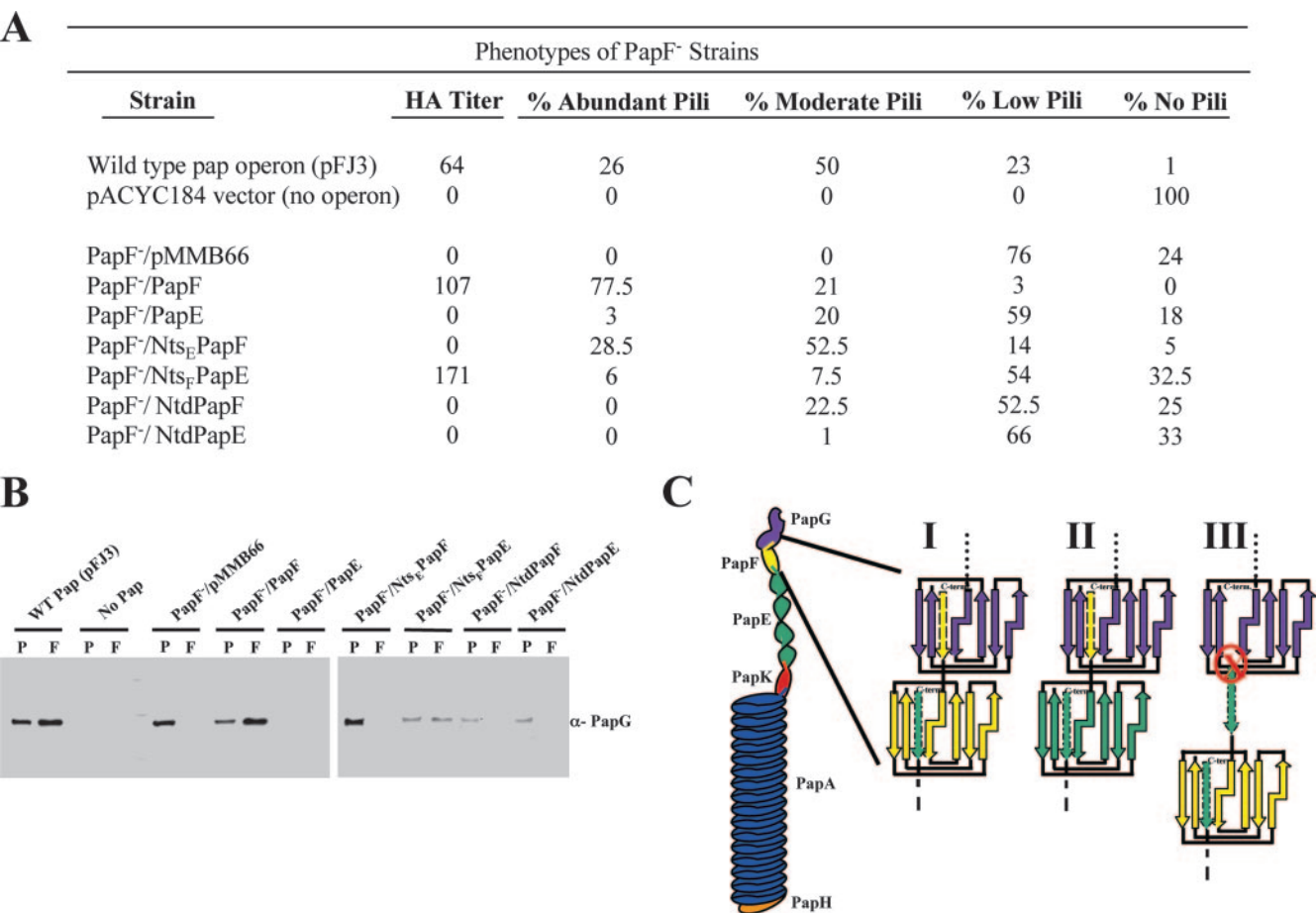


FIG. 2. HA in PapF<sup>-</sup> strains requires the PapF N-terminal extension. (A) HA and piliation experiments with PapF<sup>-</sup> strains. The ability to agglutinate red blood cells was measured in PapF<sup>-</sup> strains to which various forms of pilus subunits or an empty vector was added in *trans*. HA titers are expressed as the highest dilutions of the cell suspensions that produced detectable HA. The HA titers are the averages for three experiments. Piliation was measured by determining the numbers of piliated cells observed by negative-stain electron microscopy. Cells were categorized into four groups: no pili (bald), low numbers of pili (<20 pili per cell), moderate numbers of pili (intermediate levels), and abundant pili (high levels). A total of 200 cells were counted per experiment, and the percentages of the total falling in these four categories are shown. The data are the data from a single experiment and are representative of the trends observed in three or more experiments. (B) Presence of PapG in periplasmic and pilus preparations. The presence of PapG in periplasms and in pilus fibers produced by PapF<sup>-</sup> strains to which various mutants of PapF and PapE were added was determined in immunoblots probed with anti-PapG1 antibody, followed by development with alkaline phosphatase. Lanes P, periplasm; lanes F, pilus fibers. WT, wild type. (C) Schematic diagram of allowed subunit-subunit interactions, comparing the behavior of wild-type and Nts subunits. (I) PapG and wild-type PapF; (II) PapG and Nts<sub>E</sub>PapE; (III) PapG and Nts<sub>E</sub>PapF. The N-terminal extension of PapF (yellow) specifically interacts with the PapG pilin body (purple) (I and II), while interactions between the N-terminal extension of PapE (green) and the PapG pilin body are not allowed (III). Instead, the PapE N-terminal extension interacts with the pilin body of PapF or PapE.

positive HA titer, while cells with only the empty vector gave no titer (Fig. 2A). Similar to the no-operon vector control, PapF<sup>-</sup> bacteria to which the parent vector pMMB66 was added were also unable to agglutinate red blood cells, yielding HA titers of 0 (Fig. 2A). These results are consistent with PapF<sup>-</sup> studies conducted previously in our laboratory (21). In contrast, PapF<sup>-</sup> strains complemented with wild-type PapF demonstrated a restored ability to hemagglutinate (Fig. 2A). Positive HA correlated with the presence of PapGI in the pilus fibers, as determined by immunoblot analysis using anti-PapGI pilin antibody (Fig. 2B). While PapG was found in the periplasm of the PapF<sup>-</sup> strain carrying an empty vector, it was not observed in the pilus fiber (Fig. 2B). In contrast, abundant PapG was observed in the periplasm and

pilus fiber upon complementation of the PapF<sup>-</sup> strain with PapF (Fig. 2B). If PapF follows PapG in the mature pilus fiber, then the PapF N-terminal extension should be required to complete the Ig-like fold of the PapG pilin body in donor strand exchange, covering the hydrophobic groove caused by the absence of the final beta strand in PapG and consequently enabling proper presentation for PapG-mediated agglutination of red blood cells (Fig. 2C). To test the involvement of the specific N-terminal extension of PapF in the interaction with PapG, the levels of HA in response to production of Nts<sub>E</sub>PapE in the PapF<sup>-</sup> strain were analyzed. Similar to strains with wild-type PapF, strains complemented with Nts<sub>E</sub>PapE, which carries the N-terminal extension of PapF on the pilin body of PapE, re-

sulted in the presence of PapG in pilus preparations and exhibited positive HA (Fig. 2A and 2B). To determine if the presence of the PapE pilin body also contributed to the ability of Nts<sub>E</sub>PapE to interact with PapG, two PapE subunits, wild-type PapE and NtdPapE, were tested. Both subunits were unable to complement the PapF<sup>-</sup> strain in HA assays (Fig. 2A), and no PapG was observed in pilus preparations when an immunoblot assay was performed (Fig. 2B). Consistent with these results, replacement of only the PapF N-terminal extension with that of PapE on the pilin body of PapF, to form Nts<sub>E</sub>PapF, did not yield a subunit capable of substituting for wild-type PapF in the HA assay or in localization of PapG in pili, despite normal levels of PapG in the periplasm (Fig. 2A and 2B). Production of this Nts<sub>E</sub>PapF protein in the PapF<sup>-</sup> strain resulted in a titer of 0 (Fig. 2A), despite the presence of the PapF pilin body. Likewise, removal of the PapF N-terminal extension, leaving only the pilin body of PapF (NtdPapF), resulted in an inability of the new subunit to complement the PapF<sup>-</sup> strain, and an HA titer of 0 was observed (Fig. 2A), showing that the PapF Ig-like fold could not establish interactions with PapG in the absence of the PapF N-terminal extension. These results suggest both that the N-terminal extension of PapE does not fit into the hydrophobic groove of the PapG pilin body and that the pilin body of PapF is not required in interactions between PapF and PapG (Fig. 2C). Taken together, the results of these PapF<sup>-</sup> complementation studies show that the adaptor function of PapF is dependent upon the N-terminal extension of PapF and not the specific pilin body of PapF.

**The N-terminal extension mediates specificity and facilitates subunit arrangement via donor strand exchange.** In the PapF<sup>-</sup> studies described above, the adaptor function of PapF was demonstrated to be dependent on the specific PapF N-terminal extension through its participation in interactions with the pilin body of PapG via donor strand exchange, facilitating the proper arrangement of the two subunits in the pilus fiber (Fig. 2C). Therefore, if PapF connects PapG to the rest of the pilus, then rather than participation in interactions with PapG, the requirement for the pilin body of PapF in pilus biogenesis may be to form specific interactions with the N-terminal extension of PapE (Fig. 2C). In turn, the PapE pilin body would form interactions with the N-terminal extension of another PapE subunit or of a PapK subunit; PapK would then interact with PapA, resulting in the previously described order of subunits. To investigate the role of donor strand exchange in mediating the arrangement of all of the subunits in the pilus, Ntd and Nts subunits were produced in a PapE<sup>-</sup> strain, and the abilities of the resultant bacteria to agglutinate red blood cells were monitored. The PapE<sup>-</sup> strain was constructed like the PapF<sup>-</sup> strain, by transforming a plasmid, pYML76, containing a *papE* mutant operon under control of the natural promoter (33), into *E. coli* MC4100. Wild-type PapE, wild-type PapF, NtdPapE, NtdPapF, Nts<sub>E</sub>PapE, Nts<sub>E</sub>PapF, Nts<sub>E</sub>PapK, or the empty vector (pMMB66) was then added in *trans*. PapE<sup>-</sup> strains to which pMMB66 was added were unable to agglutinate red blood cells, yielding HA titers of 0 (Table 2), consistent with PapE<sup>-</sup> studies conducted previously (21). In contrast, PapE<sup>-</sup> strains complemented with wild-type PapE showed positive HA titers (Table 2).

Previous studies have placed repeating subunits of PapE

TABLE 2. HA in PapE<sup>-</sup> strains

Strain	HA titer <sup>a</sup>
Wild-type <i>pap</i> operon (pFJ3).....	16
pACYC184 vector (no operon).....	1
PapE <sup>-</sup> /pMMB66.....	0
PapE <sup>-</sup> /PapE.....	8
PapE <sup>-</sup> /Nts <sub>E</sub> PapF.....	0
PapE <sup>-</sup> /Nts <sub>E</sub> PapK.....	13
PapE <sup>-</sup> /Nts <sub>E</sub> PapE.....	27
PapE <sup>-</sup> /NtdPapE.....	0
PapE <sup>-</sup> /PapF.....	1
PapE <sup>-</sup> /NtdPapF.....	1

<sup>a</sup> HA titers are expressed as the highest dilution of the cell suspension that produced detectable HA. The HA titers are the averages for three experiments.

between PapF and PapK in the final pilus architecture (Fig. 1A) (21, 25). To determine the requirement for the PapE N-terminal extension for connecting PapF to the rest of the pilus fiber, the levels of HA in response to production of Nts<sub>E</sub>PapF or Nts<sub>E</sub>PapK in the PapE<sup>-</sup> strain were analyzed. Nts<sub>E</sub>PapF was unable to restore HA ability to the PapE<sup>-</sup> strain (Table 2). However, Nts<sub>E</sub>PapK was able to complement the PapE<sup>-</sup> strain, such that HA titers equivalent to those of strains with the wild-type *pap* operon were observed (Table 2). Nts<sub>E</sub>PapF and Nts<sub>E</sub>PapK both carry the PapE N-terminal extension, but they differ in the pilin body, suggesting that while the N-terminal extension of PapE could interact with a preceding PapF subunit, the difference in the abilities to complement the PapE<sup>-</sup> strain resulted from the difference in the pilin body. The failure of Nts<sub>E</sub>PapF to bridge PapF to PapK therefore suggests that the PapF pilin body could not accommodate the N-terminal extension of an incoming PapK subunit. In contrast, the PapK pilin body of Nts<sub>E</sub>PapK was able to interact with the N-terminal extension of an incoming PapA subunit. These results directly confirm previously reported orders of subunits in the mature pilus fiber and demonstrate the requirement for specific N-terminal extensions in mediating specificity between subunit-subunit interactions.

Complementation of the PapE<sup>-</sup> strain by Nts<sub>E</sub>PapK and not by Nts<sub>E</sub>PapF demonstrated the specificities of interactions between PapF and PapE and between PapK and PapA, resulting in the proper arrangement of subunits in the pilus (Fig. 1A). To investigate the requirement for the PapE pilin body in mediating interactions between PapE and PapK through donor strand exchange, Nts<sub>E</sub>PapE was tested in the HA assay. Nts<sub>E</sub>PapE was able to complement the PapE<sup>-</sup> strain, producing positive HA titers (Table 2). The presence of the N-terminal extension of PapF in Nts<sub>E</sub>PapE suggests either that PapF subunits may have some ability to interact with one another or, more likely, that overexpression of Nts<sub>E</sub>PapE from the *P*<sub>tac</sub> promoter allowed it to outcompete native PapF produced from the natural promoter in the *papE* mutant operon for interactions with PapG. Irrespective of these possibilities, these results also show that interactions between PapE and PapK required the PapE pilin body, placing PapE before PapK in the pilus fiber, as previously described. Finally, NtdPapE, wild-type PapF, and NtdPapF were also examined. NtdPapE, wild-type PapF, and NtdPapF were each unable to confer the ability to agglutinate red blood cells to the PapE<sup>-</sup> strain (Table 2). Taken together, these results suggest that the N-terminal extension facilitates the



proper order of subunits in the pilus fiber through specific interactions with pilin bodies of neighboring subunits.

**The PapF pilin body is required for nucleation of P-pilus assembly.** The HA studies described above demonstrated that the PapF N-terminal extension is necessary for PapF to function as a PapG adaptor, dictating the arrangement of subunits through donor strand exchange. It has also been previously noted that PapF may play a role in the initiation of assembly of the remaining subunits into the fiber (21). The basis for this initiator function is unclear. To directly examine the involvement of the N-terminal extension and the pilin body in the initiation of pilus assembly by PapF, relative piliation levels of PapF<sup>-</sup> strains were compared when wild-type or mutant PapF and PapE subunits were expressed in *trans*. As in the HA assays, strains MC4100/pFJ3 (wild-type *pap* operon) and MC4100/pACYC184 (empty vector) were used as positive and negative controls, respectively, for comparison. Piliation in populations of 200 bacterial cells per strain was characterized and quantitated using negative-stain electron microscopy. In addition, levels of piliation were also demonstrated by visualization of PapA via SDS-PAGE of pilus preparations followed by Coomassie blue staining.

Cells carrying the wild-type *pap* operon produced abundant pili, with the majority of the bacterial cells showing moderate to high levels of piliation (Fig. 2A and 3A, panel I). Cells with only the empty vector were bald and devoid of pili (Fig. 2A and 3A, panel II). PapF<sup>-</sup> strains to which pMMB66 was added exhibited a strong defect in pilus assembly, producing low levels of pili or no pili (Fig. 2A and 3A, panel III). As shown in Fig. 2A and 3A, panel IV, in PapF<sup>-</sup> strains complemented with wild-type PapF, piliation was restored to wild-type (i.e., whole *pap* operon) levels, with pili abundant on the cell surface. Consistent with these electron microscopy results, the levels of PapA in pili isolated from these PapF<sup>-</sup> strains were higher in the presence of PapF than in the absence of PapF, similar to results obtained for control strains carrying the wild-type *pap* operon or no operon (Fig. 3B).

To determine if the specific N-terminal extension of PapF was necessary for nucleation of pilus assembly, piliation was assessed by electron microscopy and isolation of pili in the PapF<sup>-</sup> strain after addition of the subunit Nts<sub>E</sub>PapE. In the HA assays described above, Nts<sub>E</sub>PapE was able to restore the ability to hemagglutinate in the PapF<sup>-</sup> strain (Fig. 2A). In contrast, the same subunit, Nts<sub>E</sub>PapE, was not able to restore piliation to wild-type levels in the PapF<sup>-</sup> strain, and only a small number of cells exhibited moderate to high levels of pili (Fig. 2A and 3A, panel V). Likewise, wild-type PapE and NtdPapE controls yielded mostly cells with low numbers of pili or no pili (Fig. 2A and 3A, panels VI and VII). In agreement with the electron microscopy results, only small amounts of PapA were observed in pili prepared from all three of these strains (Fig. 3B). These results suggest that while the specific PapF N-terminal extension was required to interact with PapG, it alone was not specifically responsible for PapF's role as an initiator of pilus assembly.

If the specific N-terminal extension of PapF was not required for initiation of pilus assembly, the specific pilin body may be necessary for this function. Thus, piliation was measured in PapF<sup>-</sup> strains producing Nts<sub>E</sub>PapF or NtdPapF, two proteins which contain the same PapF pilin body but differ

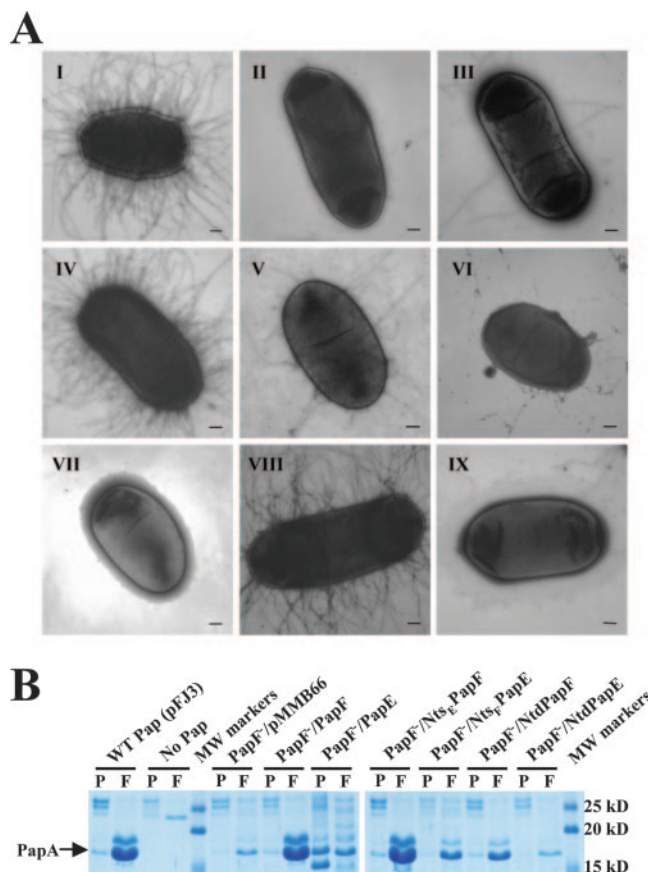


FIG. 3. Complementation of PapF<sup>-</sup> strains in pilus assembly initiation is dependent on the specific PapF incomplete Ig-like fold. (A) Electron microscopy images showing Pap piliation. PapF<sup>-</sup> strains to which various forms of pilus subunits or an empty vector was added in *trans* were examined by negative-stain electron microscopy. The micrographs show the predominant species (piliated or nonpiliated) in each sample. All images are from a single experiment, and the results are representative of three or more experiments. Bars = 200 nm. The following strains are shown: panel I, wild-type *pap* operon under control of the natural promoter (MC4100/pFJ3); panel II, no *pap* genes (MC4100/pACYC184); panel III, PapF<sup>-</sup>/empty vector (MC4100/pFJ9/pMMB66); panel IV, PapF<sup>-</sup>/PapF (MC4100/pFJ9/pYML43); panel V, PapF<sup>-</sup>/Nts<sub>E</sub>PapE (MC4100/pFJ9/pYML56); panel VI, PapF<sup>-</sup>/PapE (MC4100/pFJ9/pHJ13); panel VII, PapF<sup>-</sup>/NtdPapE (MC4100/pFJ9/pYML36); panel VIII, PapF<sup>-</sup>/Nts<sub>E</sub>PapF (MC4100/pFJ9/pYML55); panel IX, PapF<sup>-</sup>/NtdPapF (MC4100/pFJ9/pYML64). (B) SDS-PAGE of periplasm and pili produced by PapF<sup>-</sup> strains. Periplasm was extracted by the lysozyme-EDTA method as described previously and boiled prior to loading. Pili were removed from bacterial cells by heat extraction and exposed to boiling and 4 M urea to separate subunits. Levels of PapA (arrow) in the periplasm (lanes P) and in the pilus fibers (lanes F) were visualized following Coomassie blue staining. WT, wild type; MW, molecular weight.

by the presence or absence of an N-terminal extension. The presence of the N-terminal extension of PapE on the PapF pilin body (Nts<sub>E</sub>PapF) increased the levels of piliation in PapF<sup>-</sup> strains, enabling more than one-half of the observed PapF<sup>-</sup> cells to produce abundant pili (Fig. 2A and 3A, panel VIII). Likewise, high levels of PapA were also determined by SDS-PAGE of pili prepared from the PapF<sup>-</sup>/Nts<sub>E</sub>PapF strains (Fig. 3B). Lower levels of piliation were observed in PapF<sup>-</sup> strains to which NtdPapF was added (Fig. 2A, 3A, panel IX,



and 3B). Notably, however, these results are in contrast to the results of the HA studies described above, as neither NtdPapF nor Nts<sub>E</sub>PapF was able to complement the PapF<sup>-</sup> strain in HA assays (Fig. 2A), consistent with the requirement of the specific PapF N-terminal extension to adapt PapG to the pilus. These results show that the specific pilin body of PapF was essential to initiate pilus assembly and that both a PapF N-terminal extension and a PapE N-terminal extension could facilitate this function.

## DISCUSSION

Assembly of P pili occurs via the chaperone-usher pathway, a highly conserved pathway that is used in the construction of more than 100 other adhesive organelles in a wide range of diverse gram-negative bacteria (15, 19; unpublished data). The P pilus is a prototypical example of these various adhesive organelles (16, 43). All structural subunits in P pilus share the same overall topology (39, 40). Thus, the subunits could possibly all have the same unilateral function; i.e., they could act as simple building blocks within the final pilus fiber. However, through examination of the PapF structure and function, we report in this study a more complex role of the different pilus subunits in pilus biogenesis and show that there may be significant differences in function between structurally similar subunits.

In this work, Ntd and Nts subunits were used to demonstrate that the ordering of the subunit types in the pilus is determined by the donor strand exchange reaction involving specific N-terminal extensions. The Ntd and Nts subunits were used because they enabled examination of each domain of the pilus subunits: the N-terminal extension and the pilin body. We found that in a PapF<sup>-</sup> strain, the N-terminal extension of PapF was required for adapting PapG to the pilus tip. As shown in experiments with Nts<sub>E</sub>PapE, even in the absence of the PapF pilin body, PapG was adapted to the pilus tip as long as the PapF N-terminal extension was presented. Since PapG is the first subunit incorporated into a growing pilus at the outer membrane usher, the N-terminal extension of PapF must interact with the pilin domain of PapG, presumably by completing the Ig-like fold during donor strand exchange. Consistent with these results was the inability of the Nts<sub>E</sub>PapF subunit to adapt PapG to the pilus tip, as determined in the HA assay. These results show directly that the ability of PapF to interact specifically with PapG is dependent only on the N-terminal extension.

In order to complement a PapE<sup>-</sup> strain and restore HA titers, a minimum of two requirements must be met: (i) an N-terminal extension must be available for interaction with the pilin body of PapF and (ii) an appropriate pilin body must be present for interaction with the N-terminal extension of an incoming pilus subunit. The subunits Nts<sub>E</sub>PapE and Nts<sub>E</sub>PapK both fulfilled each of these requirements. Nts<sub>E</sub>PapE, carrying the PapF N-terminal extension, presumably competes with wild-type PapF for interaction with PapG and, in contrast to PapF, possesses the PapE Ig-like domain, enabling it to interact with the appropriate incoming subunit. Restoration of HA titers in the PapE<sup>-</sup> strain by Nts<sub>E</sub>PapK was allowed because the presence of the N-terminal extension of PapE permitted Nts<sub>E</sub>PapK to interact with PapF, while simultaneous interac-

tion with PapA was possible due to the presence of the pilin body of PapK. These experiments thus confirm the following order for pilus subunit incorporation into the fiber, mediated by the N-terminal extensions: PapG, PapF, PapE, PapK, PapA (Fig. 1A).

It has been previously shown that *papF* mutants produce few or no pili, suggesting that PapF may also act as an initiator of pilus assembly (21). We discovered that the adaptor function of PapF, mediated by the N-terminal extension, could not fully account for this putative initiator function. Further, while the specific N-terminal extension of PapF is not required for this initiator function, the pilin body of PapF must be presented in the context of an N-terminal extension for efficient initiation, as reflected by the highly diminished ability of NtdPapF to promote pilus assembly. How PapF controls pilus assembly is currently not understood; however, it may aid the usher PapC in its transition into an assembly-competent state. Saulino et al. demonstrated a shift in the usher from a closed state to an assembly-competent state in the related *fim* system upon binding to the adhesin, which in the *fim* system also serves as an initiator of pilus assembly (41). In the *fim* system, binding of all nonadhesin chaperone-subunit complexes depended on the initial binding of the FimH adhesin-chaperone complex, which induced the usher shift, possibly explaining the additional role of FimH in initiation of pilus assembly (41). A similar shift has not been reported for PapC in the P-pilus system, possibly because PapF may assist the adhesin in this process. Unlike in the *fim* system, in the P-pilus system, usher binding by the subunit-chaperone complex that immediately follows the adhesin, PapF-PapD, is not dependent on prior binding by the PapG-PapD adhesin-chaperone complex (10). Instead, PapF-PapD complexes, like PapG-PapD adhesin-chaperone complexes, have been shown to independently bind to the usher in *in vitro* assays (10). We thus argue that in addition to events that may be mediated by PapG, the initiation event in P-pilus biogenesis also requires an interaction with PapC involving the PapF pilin body.

In conclusion, by using a combination of Ntd and Nts subunits, we were able to dissect the adaptor and nucleator functions of PapF and assign separate domains of the protein to the different functions. We discovered that the specific PapF N-terminal extension played an important role in interactions with PapG, while at the same time, it facilitated the ability of the Ig-like domain to initiate pilus assembly. The pilin body likewise had dual functions in the cell, working in part to facilitate pilus assembly by playing an active role in the biogenesis pathway, while at the same time providing an interactive surface for binding with subsequent subunits.

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## REFERENCES

- Baga, M., M. Norgren, and S. Normark. 1987. Biogenesis of *E. coli* Pap pili: PapH, a minor pilin subunit involved in cell anchoring and length modulation. *Cell* **49**:241–251.
- Baga, M., S. Normark, J. Hardy, P. O'Hanley, D. Lark, O. Olsson, G. Schoolnik, and S. Falkow. 1984. Nucleotide sequence of the *papA* gene encoding the Pap pilus subunit of human uropathogenic *Escherichia coli*. *J. Bacteriol.* **157**:330–333.
- Barnhart, M. M., J. S. Pinkner, G. E. Soto, F. G. Sauer, S. Langermann, G. Waksman, C. Frieden, and S. J. Hultgren. 2000. PapD-like chaperones provide the missing information for folding of pilin proteins. *Proc. Natl. Acad. Sci. USA* **97**:7709–7714.
- Barnhart, M. M., F. G. Sauer, J. S. Pinkner, and S. J. Hultgren. 2003. Chaperone-subunit-usher interactions required for donor strand exchange during bacterial pilus assembly. *J. Bacteriol.* **185**:2723–2730.
- Bock, K., M. E. Breimer, A. Brignole, G. C. Hansson, K.-A. Karlsson, G. Larson, H. Leffler, B. E. Samuelsson, N. Strömberg, C. Svanborg-Edén, and J. Thulin. 1985. Specificity of binding of a strain of uropathogenic *Escherichia coli* to Gal $\alpha$ (1-4)Gal-containing glycosphingolipids. *J. Biol. Chem.* **260**:8545–8551.
- Bullitt, E., C. H. Jones, R. Striker, G. Soto, F. Jacob-Dubuisson, J. Pinkner, M. J. Wick, L. Makowski, and S. J. Hultgren. 1996. Development of pilus organelle subassemblies *in vitro* depends on chaperone uncapping of a beta zipper. *Proc. Natl. Acad. Sci. USA* **93**:12890–12895.
- Bullitt, E., and L. Makowski. 1995. Structural polymorphism of bacterial adhesion pili. *Nature* **373**:164–167.
- Casadaban, M. J. 1976. Transposition and fusion of *lac* genes to selected promoters in *Escherichia coli* using bacteriophages *lambda* and *Mu*. *J. Mol. Biol.* **104**:541–555.
- Choudhury, D., A. Thompson, V. Sojanoff, S. Langermann, J. Pinkner, S. J. Hultgren, and S. Knight. 1999. X-ray structure of the FimC-FimH chaperone-adhesin complex from uropathogenic *Escherichia coli*. *Science* **285**:1061–1065.
- Dodson, K. W., F. Jacob-Dubuisson, R. T. Striker, and S. J. Hultgren. 1993. Outer membrane PapC usher discriminately recognizes periplasmic chaperone-pilus subunit complexes. *Proc. Natl. Acad. Sci. USA* **90**:3670–3674.
- Dodson, K. W., J. S. Pinkner, T. Rose, G. Magnusson, S. J. Hultgren, and G. Waksman. 2001. Structural basis of the interaction of the pyelonephritic *E. coli* adhesin to its human kidney receptor. *Cell* **105**:733–743.
- Furste, J. P., W. Pansegrau, R. Frank, H. Blocker, P. Scholz, M. Bagdasarian, and E. Lanka. 1986. Molecular cloning of the plasmid RP4 primase region in a multi-host-range *lacP* expression vector. *Gene* **48**:119–131.
- Gong, M., and L. Makowski. 1992. Helical structure of Pap adhesion pili from *Escherichia coli*. *J. Mol. Biol.* **228**:735–742.
- Holmgren, A., and C. Brändén. 1989. Crystal structure of chaperone protein PapD reveals an immunoglobulin fold. *Nature* **342**:248–251.
- Hultgren, S. J., F. Jacob-Dubuisson, C. H. Jones, and C.-I. Brändén. 1993. PapD and superfamily of periplasmic immunoglobulin-like pilus chaperones. *Adv. Protein Chem.* **44**:99–123.
- Hultgren, S. J., C. H. Jones, and S. N. Normark. 1996. Bacterial adhesins and their assembly, p. 2730–2756. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, DC.
- Hultgren, S. J., F. Lindberg, G. Magnusson, J. Kihlberg, J. M. Tennent, and S. Normark. 1989. The PapG adhesin of uropathogenic *Escherichia coli* contains separate regions for receptor binding and for the incorporation into the pilus. *Proc. Natl. Acad. Sci. USA* **86**:4357–4361.
- Hung, D. L., S. D. Knight, and S. J. Hultgren. 1999. Probing conserved surfaces on PapD. *Mol. Microbiol.* **31**:773–783.
- Hung, D. L., S. D. Knight, R. M. Woods, J. S. Pinkner, and S. J. Hultgren. 1996. Molecular basis of two subfamilies of immunoglobulin-like chaperones. *EMBO J.* **15**:3792–3805.
- Hung, D. L., J. S. Pinkner, S. D. Knight, and S. J. Hultgren. 1999. Structural basis of chaperone self-capping in P pilus biogenesis. *Proc. Natl. Acad. Sci. USA* **96**:8178–8183.
- Jacob-Dubuisson, F., J. Heuser, K. Dodson, S. Normark, and S. J. Hultgren. 1993. Initiation of assembly and association of the structural elements of a bacterial pilus depend on two specialized tip proteins. *EMBO J.* **12**:837–847.
- Jones, C. H., P. N. Danese, J. S. Pinkner, T. J. Silhavy, and S. J. Hultgren. 1997. The chaperone-assisted membrane release and folding pathway is sensed by two signal transduction systems. *EMBO J.* **16**:6394–6406.
- Jones, C. H., K. Dodson, and S. J. Hultgren. 1996. Structure, function, and assembly of adhesive P pili, p. 175–219. *In* H. L. T. Mobley and J. W. Warren (ed.), *Urinary tract infections: molecular pathogenesis and clinical management*, 1st ed. ASM Press, Washington, DC.
- Justice, S. S., D. A. Hunstad, J. R. Harper, A. R. Duguay, J. S. Pinkner, J. Bann, C. Frieden, T. J. Silhavy, and S. J. Hultgren. 2005. Periplasmic peptidyl prolyl *cis-trans* isomerases are not essential for viability, but SurA is required for pilus biogenesis in *Escherichia coli*. *J. Bacteriol.* **187**:7680–7686.
- Kuehn, M. J., J. Heuser, S. Normark, and S. J. Hultgren. 1992. P pili in uropathogenic *E. coli* are composite fibres with distinct fibrillar adhesive tips. *Nature* **356**:252–255.
- Kuehn, M. J., S. Normark, and S. J. Hultgren. 1991. Immunoglobulin-like PapD chaperone caps and uncaps interactive surfaces of nascently translocated pilus subunits. *Proc. Natl. Acad. Sci. USA* **88**:10586–10590.
- Kuehn, M. J., D. J. Ogg, J. Kihlberg, L. N. Slonim, K. Flemmer, T. Bergfors, and S. J. Hultgren. 1993. Structural basis of pilus subunit recognition by the PapD chaperone. *Science* **262**:1234–1241.
- Lee, Y. M., P. A. DiGiuseppe, T. J. Silhavy, and S. J. Hultgren. 2004. P pilus assembly motif necessary for activation of the CpxRA pathway by PapE in *Escherichia coli*. *J. Bacteriol.* **186**:4326–4337.
- Leffler, H., and C. Svanborg-Edén. 1980. Chemical identification of a glycosphingolipid receptor for *Escherichia coli* attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. *FEMS Microbiol. Lett.* **8**:127–134.
- Li, H., L. Qian, Z. Chen, D. Thibault, G. Liu, T. Liu, and D. G. Thanassi. 2004. The outer membrane usher forms a twin-pore secretion complex. *J. Mol. Biol.* **344**:1397–1407.
- Lindberg, F., B. Lund, L. Johansson, and S. Normark. 1987. Localization of the receptor-binding protein adhesin at the tip of the bacterial pilus. *Nature* **328**:84–87.
- Lindberg, F. P., B. Lund, and S. Normark. 1986. Gene products specifying adhesion of uropathogenic *Escherichia coli* are minor components of pili. *Proc. Natl. Acad. Sci. USA* **83**:1891–1895.
- Lindberg, F. P., B. Lund, and S. Normark. 1984. Genes of pyelonephritogenic *E. coli* required for digalactoside-specific agglutination of human cells. *EMBO J.* **3**:1167–1173.
- Lund, B., F. Lindberg, B. I. Marklund, and S. Normark. 1987. The PapG protein is the alpha-D-galactopyranosyl-(1-4)-beta-D-galactopyranose-binding adhesin of uropathogenic *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **84**:5898–5902.
- Marklund, B. I., J. M. Tennent, E. Garcia, A. Hamers, M. Baga, F. Lindberg, W. Gastra, and S. Normark. 1992. Horizontal gene transfer of the *Escherichia coli* *pap* and *prs* pili operons as a mechanism for the development of tissue-specific adhesive properties. *Mol. Microbiol.* **6**:2225–2242.
- Mulvey, M. A. 2002. Adhesion and entry of uropathogenic *Escherichia coli*. *Cell. Microbiol.* **4**:257–271.
- Mulvey, M. A., J. D. Schilling, J. J. Martinez, and S. J. Hultgren. 2000. Bad bugs and beleaguered bladders: interplay between uropathogenic *Escherichia coli* and innate host defenses. *Proc. Natl. Acad. Sci. USA* **97**:8829–8835.
- Roberts, J. A., B.-I. Marklund, D. Ilver, D. Haslam, M. B. Kaack, G. Baskin, M. Louis, R. Mollby, J. Winberg, and S. Normark. 1994. The Gal  $\alpha$ (1-4) Gal-specific tip adhesin of *Escherichia coli* P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract. *Proc. Natl. Acad. Sci. USA* **91**:11889–11893.
- Sauer, F. G., K. Futterer, J. S. Pinkner, K. W. Dodson, S. J. Hultgren, and G. Waksman. 1999. Structural basis of chaperone function and pilus biogenesis. *Science* **285**:1058–1061.
- Sauer, F. G., J. S. Pinkner, G. Waksman, and S. J. Hultgren. 2002. Chaperone priming of pilus subunits facilitates a topological transition that drives fiber formation. *Cell* **111**:543–551.
- Saulino, E. T., D. G. Thanassi, J. Pinkner, and S. J. Hultgren. 1998. Ramifications of kinetic partitioning on usher-mediated pilus biogenesis. *EMBO J.* **17**:2177–2185.
- Stromberg, N., B. I. Marklund, B. Lund, D. Ilver, A. Hamers, W. Gastra, K. A. Karlsson, and S. Normark. 1990. Host-specificity of uropathogenic *Escherichia coli* depends on differences in binding specificity to Gal $\alpha$ (1-4)Gal-containing isoreceptors. *EMBO J.* **9**:2001–2010.
- Thanassi, D. G., E. T. Saulino, and S. J. Hultgren. 1998. The chaperone/usher pathway: a major terminal branch of the general secretory pathway. *Curr. Opin. Microbiol.* **1**:223–231.
- Thanassi, D. G., E. T. Saulino, M.-J. Lombardo, R. Roth, J. Heuser, and S. J. Hultgren. 1998. The PapC usher forms an oligomeric channel: implications for pilus biogenesis across the outer membrane. *Proc. Natl. Acad. Sci. USA* **95**:3146–3151.
- Wertman, K. F., A. R. Wyman, and D. Botstein. 1986. Host/vector interactions which affect the viability of recombinant phage *lambda* clones. *Gene* **49**:253–262.